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# Protective effect of recombinant human glucagon-like peptide-1 (rhGLP-1) pretreatment in STZ-induced diabetic mice

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**Human glucagon-like peptide-1 (hGLP-1) and its mimetics have emerged as therapies for type 2 diabetes. However, clinical treatment of diabetes with hGLP-1 is ineffective because of rapid DPPIV-mediated hGLP-1 degradation in the circulation. In this study, we investigated the protective effect of recombinant human glucagon-like peptide-1 (rhGLP-1) treatment on STZ-induced diabetic mice. Mice were treated daily with rhGLP-1 (24 nmol/kg body weight) starting before or after STZ injection (40 mg/kg body weight) to induce diabetes. Mice pretreated with rhGLP-1 before but not after STZ showed significantly reduced blood glucose levels ($P < 0.05$), increased oral glucose tolerance (area under the curve, $1740 \pm 71.18$ vs $2416 \pm 205.6$, $P < 0.05$). Furthermore, the bioproduct of lipid peroxidation, MDA, was reduced and SOD and GSH-PX activities were enhanced globally and in pancreas of mice that received rhGLP-1 pretreatment before STZ, when comparing with STZ-treated mice. Finally, STZ-induced pancreatic islet damage was rescued by rhGLP-1 pretreatment. Taken together, the results of this study demonstrate that rhGLP-1 pretreatment has a protective effect against STZ-induced diabetes in mice. These findings suggest that the GLP-1 pretreatment may be a new therapeutic strategy in the preventive and protective treatment during diabetes initiation and progression.** 

*Supporting information may be found in the online version of this article*

**Keywords:** GLP-1; streptozotocin; lipid peroxidation; oxidative stress; diabetes mellitus; type 2 diabetes

## Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia [1]. Functional impairment of pancreas beta cells caused by reactive oxygen species (ROS) contributes to the increasing blood glucose in both type I and type II diabetes. ROS production is stimulated by growth factor withdrawal, human immunodeficiency virus infection, and cytokines including tumor necrosis factor $\alpha$ (TNF-$\alpha$) and lipopolysaccharide [2,3]. ROS induce cellular damages including lipid peroxidation, protein oxidation, and DNA damage, all of which cause beta cell insufficiency and eventual death. In contrast, overexpression of antioxidant enzyme SOD or anti-apoptotic protein Bcl-2 can inhibit ROS-induced apoptosis [4,5]. These findings indicate ameliorating oxidative stress and beta cell damage may provide benefits to a broad subset of diabetic patients.

Human glucagon-like peptide-1 (hGLP-1) has been shown to improve glucose-dependent insulin secretion, reduce plasma glucagon level, and inhibit gluconeogenesis [6]. However, hGLP-1 has a short half-life (less than 2 min) because of rapid DPPIV-mediated degradation *in vivo*. Therefore, once-daily hGLP-1 in the treatment of diabetic patients is ineffective. Recently, an increasing number of studies have uncovered additional functions for GLP-1 and its analogs. The evidence from our previous study demonstrated that a GLP-1 analog (mGLP-1) protected SH-SY5Y cells from apoptosis induced by amyloid-beta peptide [7]. Study from Hui's [8] group showed GLP-1 treatment before the exposure to $H_2O_2$ but not after had a protective effect on $H_2O_2$-induced apoptosis in insulinoma beta cells (MIN6) via a cAMP- and PI3K-dependent pathway. This study was undertaken to investigate whether recombinant hGLP-1 (rhGLP-1) has a protective effect on STZ-induced diabetic mice *in vivo*, independently from the amelioration of insulin secretion and the acquired glucose control that follow its administration. We treated mice with rhGLP-1 starting before or after STZ injection and then examined blood

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**Abbreviations used:** *rhGLP-1, recombinant human glucagon-like peptide-1; STZ, streptozotocin; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; CAT, catalase activity; KM mice, Kunming mice; H&E, hematoxylin and eosion; SE, standard error; OGTT, oral glucose tolerance test; MDA, malondialdehyde; DPPIV, dipeptidyl peptidase IV; T1D, type 1 diabetes; T2D, type 2 diabetes TNF-α, tumor necrosis factor α; ANOVA, analysis of variance.*

 

glucose levels, glucose tolerance, pancreas islet damage, and associated changes in oxidative stress and antioxidant activities in STZ-induced diabetic mice with rhGLP-1 treatment.

## Materials and Methods

### Animal Experiments

Chinese KM mice, an outbreed strain of laboratory animal, were derived in 1944 from a pair of Swiss mice that had been introduced from Hoffline Institution of Hindustan into Kunming of China, hence the name KM mice [9]. KM mice were maintained on a standard chow diet with a 12-h day–night schedule. rhGLP-1 was cloned, expressed, and purified by our group. It has the same characteristics as normal GLP-1 which was reported in our published article [10]. At 8 weeks of age, the mice were randomly assigned to one of three treatment groups ($n = 8$ mice per treatment group), and multiple physiological parameters were examined during a 30-day experimental period. Group I (control) mice served as normal controls and received once-daily intraperitoneal injections of 0.9% NaCl solution. Group II (STZ) mice received once-daily intraperitoneal injections of STZ on days 8–10 (40 mg/kg body weight). Group III (pre-GLP-1 + STZ) mice were pretreated with rhGLP-1 before receiving STZ injections: they received once-daily intraperitoneal injections of rhGLP-1(24 nmol/kg body weight) beginning on the first day of the study and concomitant once-daily intraperitoneal injections of STZ on days 8–10 (40 mg/kg body weight). Food and water consumption were measured every 5 days.

In a separate experiment, Group IV (STZ) mice received once-daily intraperitoneal injections of STZ (40 mg/kg body weight) on days 1–3, and the mice were then followed for 17 days. Group V (STZ + post-GLP-1) mice were treated with rhGLP-1 after receiving STZ injections: they ($n = 8$ mice per treatment group) received once-daily intraperitoneal injections of STZ (40 mg/kg body weight) on days 1–3, followed by once-daily intraperitoneal injections of rhGLP-1 (24 nmol/kg body weight) on days 7–17.

### Blood Glucose Measurements and OGTT

Blood samples were taken from the tail vein of mice fasting for 4 h on alternating days over the entire experimental period, and blood glucose levels were measured using a commercial glucometer (MicroSense Inc., China). On day 30, an OGTT was conducted following an overnight (16 h) fast. Glucose (1.5 g/kg body weight) was administered by oral gavage, and tail vein blood samples were collected at 0, 5, 15, 30, 60, and 120 min post-gavage to measure blood glucose levels. Plots of blood glucose levels *versus* post-gavage time were generated using Prism 4 software (GraphPad Software Inc., USA), and the area under the curve (AUC) [11] was determined.

### Histopathological Examination of Pancreases

Pancreases were isolated immediately after sacrifice, washed with ice-cold saline, fixed overnight in 4% paraformaldehyde solution and embedded in paraffin. Seven-micron sections were cut and stained with H&E for histopathological assessment. Islet number was estimated by counting focal islets on five sections for each pancreas, each spaced 245 μm (35 sections) apart [12]. The area of each islet (μm) and the total area of the pancreas (μm) were determined for each section, as described previously [13].

### Plasma and Pancreas Oxidative Stress Marker Measurements

Mice were euthanized on day 31 by ether anesthesia overdose, and blood samples were collected by cardiac puncture. The same amount of pancreas was homogenized in 500 μl Tris–HCl (pH 7.4) and centrifuged at 3800 rpm for 5 min and the supernatant was collected. Plasma and pancreas MDA, a surrogate marker for lipid peroxidation, and antioxidant enzymes, including SOD and GSH-PX, were measured using assay kits provided by Shenneng Bocai Company (Shanghai, China).

### Statistical Analyses

To determine significance between the groups, comparisons were made using two-tailed *t*-tests. Analyses of multiple groups were performed using one-way or two-way ANOVAs (analysis of variances), followed by the Bonferroni posttest using GraphPad Prism Version 4. For all statistical tests, $P$ values $<0.05$ were accepted as statistically significant.

## Results

### Pretreatment with rhGLP-1 Lowered Blood Glucose Levels in STZ-Induced Diabetic Mice

To determine whether rhGLP-1 pretreatment protects against STZ-induced diabetes, blood glucose levels were monitored over a 30-day experimental period in three groups of mice. Group I (control) mice were treated with saline. Group II (STZ) mice were STZ-induced diabetic mice that were treated with STZ on days 8–10 and developed progressive hyperglycemia thereafter. Group III (pre-GLP-1 + STZ) mice received daily rhGLP-1 (24 nmol/kg body weight) injections throughout the 30-day experimental period with concomitant daily STZ injections on days 8–10. At the onset of the study, the morning fasting blood glucose levels were equivalent among all the three groups (~4 mmol/l) (Figure 1(A)). Blood glucose levels in Group I (control) mice did not differ significantly from baseline at any point during the 30-day experimental period. Blood glucose levels in Group II (STZ) mice were significantly higher than that in Group I (control) mice by day 15 (15 vs 5.8 mmol/l, respectively) and remained higher than 15 mmol/l for the rest of the experimental period (Figure 1(A)). Treatment of Group III mice (pre-GLP-1 + STZ) did not induce subnormal glucose levels at any time point before STZ treatment. On experimental day 15, Group III (pre-GLP-1 + STZ) mice exhibited moderately increased blood glucose levels (11 mmol/l) compared with Group I (control) mice (5.8 mmol/l); this value was significantly lower than that of Group II (STZ) mice (15 mmol/l) (Figure 1(A)). For the remainder of the experimental period, the blood glucose levels of Group III (pre-GLP-1 + STZ) mice ranged from 11 to 14 mmol/l and were significantly lower at each time point than the blood glucose levels of Group II (STZ) mice (Figure 1(A)).

Notably, results from a separate experiment with Group V (STZ + post-GLP-1) mice, which received once-daily intraperitoneal injections of STZ (40 mg/kg body weight) on days 1–3 followed by once-daily intraperitoneal injections of rhGLP-1 (24 nmol/kg body weight) on days 7–17, showed that rhGLP-1 treatment after STZ injection was not associated with decreased blood glucose levels relative to control Group IV (STZ) mice injected with STZ on days 1–3 without rhGLP-1 treatment (Figure 1(B)). These results suggested that pretreatment of GLP-1 before STZ administration but not after had capability of preventing the increased blood glucose induced by STZ.



  

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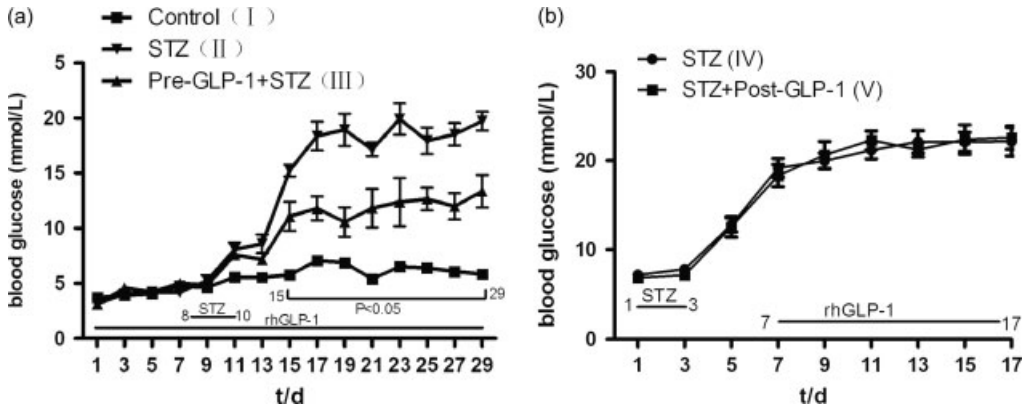


**Figure 1.** Effects of rhGLP-1 on blood glucose levels. (a) Morning fasting blood glucose levels of the three experimental groups ($n = 8$ mice per group). Group I (saline) mice were saline-injected controls. Group II (STZ) mice were administered STZ on experimental days 8–10. Group III (pre-GLP-1 + STZ) mice were administrated rhGLP-1 throughout the entire study with concomitant STZ treatment on days 8–10. Blood glucose levels were significantly lower from day 15 to 29 in Group III mice compared with blood glucose levels in Group II mice ($^*P < 0.05$). (b) Morning fasting blood glucose levels of Group IV (STZ) mice and Group V (STZ + post-GLP-1) mice ($n = 8$ mice per group).

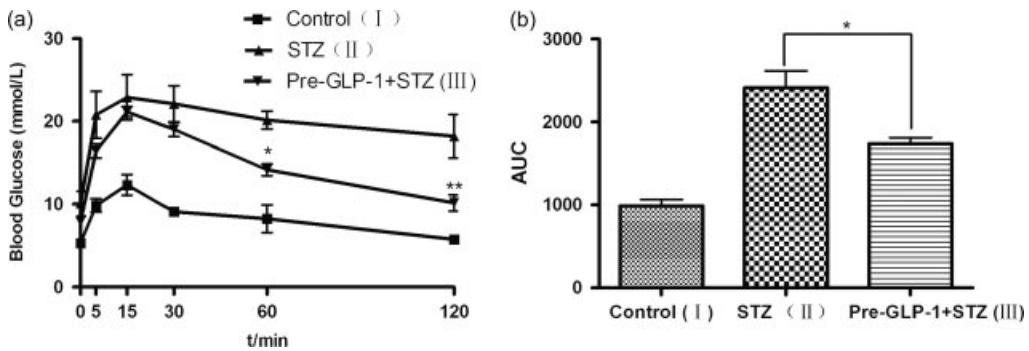


**Figure 2.** Effect of rhGLP-1 treatment on glucose tolerance: OGTT. (a) Blood glucose analysis in OGTT of mice in Group I (control), Group II (STZ), and Group III (pre-GLP-1 + STZ) mice ($n = 8$ mice per group). $^*P < 0.05$, $^{**}P < 0.01$ for differences between STZ-induced diabetic mice with or without rhGLP-1 pretreatment. (b) The AUC [11] for blood glucose levels was significantly lower in Group III mice compared with Group II mice ($^*P < 0.05$).

### Pretreatment with rhGLP-1 Improved Glucose Tolerance in STZ-Induced Diabetic Mice

To determine whether rhGLP-1 administration improved the systemic response to glucose in STZ-induced diabetic mice, OGTT was performed. On day 30, mice from Groups I, II, and III were subjected to fasting conditions for 16 h, and glucose was administered by oral gavage (1.5 g/kg). Group III (pre-GLP-1 + STZ) mice, which were pretreated with rhGLP-1, showed a significant improvement in blood glucose metabolism (Figure 2(A)). Blood glucose levels in Group II (STZ) and Group III (pre-GLP-1 + STZ) mice were significantly different in fasting mice at 60 min after glucose administration (20.1 ± 2.6 vs 14.2 ± 1.7 mmol/l, respectively). The differences in blood glucose levels between Group II and III mice persisted from 60 to 120 min after glucose administration (18.2 ± 6.4 vs 10.2 ± 2.4 mmol/l, respectively, $P < 0.01$) (Figure 2(A)). Consequently, the glucose AUC undergoing the OGTT in Group III (1740 ± 71.18) mice was lower than that in Group II (2416 ± 205.6) mice in response to rhGLP-1 treatment ($P < 0.05$) (Figure 2(B)). Therefore, in addition to improving morning fasting blood glucose levels, rhGLP-1 pretreatment significantly improved glucose tolerance in STZ-induced diabetic mice. Moreover, improvement of blood glucose levels at the 60- and 120-min time points suggested improvement in beta cell function.

### Pretreatment with rhGLP-1 Prevented Polyphagia and Polydipsia in STZ-Induced Diabetic Mice

STZ treatment induced progressive polyphagia and polydipsia in Group II (STZ) mice. On experimental day 20, which was 10 days after STZ administration, Group II mice consumed significantly more food (7.5 ± 0.6 g/mouse/day) than Group I (control) (5.9 ± 0.5 g/mouse/day) and Group III (pre-GLP-1 + STZ) (6.4 ± 0.3 g/mouse/day) mice (Figure 3(A)). These differences in food consumption persisted until the end of the experiment. On experimental day 25, Group II (STZ) mice consumed 7.96 ± 0.8 g/mouse/day, whereas Group III (pre-GLP-1 + STZ) mice consumed only 6.3 ± 0.3 g/mouse/day (Figure 3(A)). Water consumption was also significantly higher in Group II (STZ) mice than that in Group I (control) and Group III (pre-GLP-1 + STZ) mice from experimental days 10–25 (Figure 3(B)). By experimental day 25, the polydipsia in Group II (STZ) mice had progressed to such an extent that they consumed more than three times (22.9 ± 3.4 ml/mouse/day) as much water as Group I (control) (7.3 ± 0.5 ml/mouse/day) and Group III (pre-GLP-1 + STZ) (7.5 ± 1.5 ml/mouse/day) mice (Figure 3(B)). These results demonstrated that, in addition to lowering blood glucose (Figure 1), rhGLP-1 pretreatment prevented STZ-induced polyphagia and polydipsia, which are physical characteristics associated with diabetes.



  

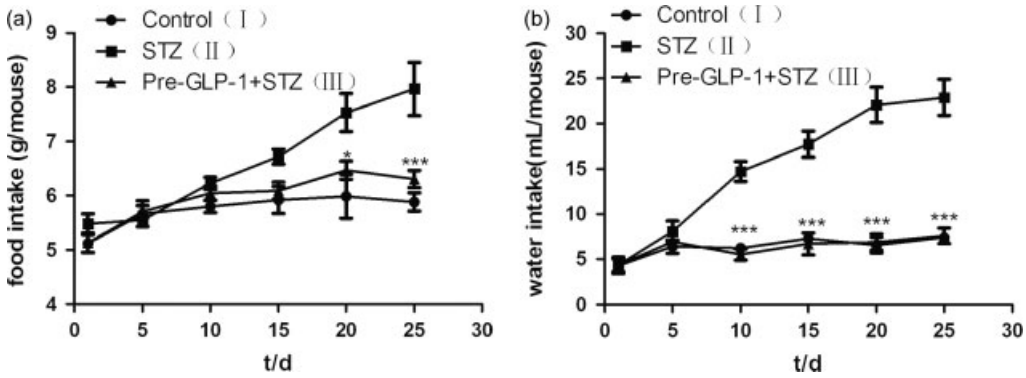


**Figure 3.** Effects of rhGLP-1 on food (a) and water (b) intake. Shown are daily food and water intake levels in Group I (control), Group II (STZ), and Group III (pre-GLP-1 + STZ) mice. $^{*}P < 0.05$, $^{***}P < 0.001$ for differences between STZ-induced diabetic mice with or without rhGLP-1 pretreatment.

### Pretreatment with rhGLP-1 Improved the Global and Pancreas Oxidative Stress Parameters in STZ-Induced Diabetic Mice

Consistent with previous reports [14], several parameters of global and pancreas oxidative stress were changed in STZ-induced diabetic mice. MDA, a bioproduct of oxidative stress-induced membrane lipid peroxidation, was significantly higher in Group II (STZ) (3.70 ± 0.50 mmol/l in plasma and 6.07 ± 0.85 mmol/l in pancreas) than in Group I (control) mice (2.09 ± 0.50 mmol/l in plasma and 3.04 ± 0.47 mmol/l in pancreas) (Tables 1 and 2). Notably, MDA level in Group III (pre-GLP-1 + STZ) mice was significantly lower (3.00 ± 0.37 mmol/l in plasma and 3.25 ± 0.82 mmol/l in pancreas) than that in Group II (STZ) mice (Tables 1 and 2). Plasma levels of SOD and nonenzymatic GSH-PX were also measured in the three groups. In plasma, Group I (control) and Group II (STZ) mice had similar SOD activities (178.56 ± 13.44 and 176.29 ± 11.06 U/ml, respectively). Unexpectedly, SOD enzymatic activity in Group III (pre-GLP-1 + STZ) mice was 197.64 ± 21.28 U/ml, which was significantly higher than the SOD enzymatic activity observed in Group I and II mice. Similarly, SOD enzymatic activity in pancreas of Group III (pre-GLP-1 + STZ) mice was increased compared with those of Group I (control) and Group II (STZ) mice, although SOD enzymatic activity in pancreas of Group II mice was lower than that of Group I mice. STZ treatment caused a significant reduction in GSH-PX activity in plasma and pancreas (261.36 ± 23.36 mg/l in plasma and 283.10 ± 28.57 mg/l in pancreas) compared with those in Group I (control) mice (304.23 ± 48.49 in plasma and 340.68 ± 32.95 in pancreas). In contrast, GSH-PX activity levels in Group III (pre-GLP-1 + STZ) mice (303.61 ± 22.13 mg/l in plasma and 304.11 ± 48.31 mg/l in pancreas) were increased compared with Group II mice, although there was no statistic difference in the GSH-PX activity between Groups II and III in pancreas. Collectively, these results suggested that pretreatment of rhGLP-1 may relax the STZ-induced lipid peroxidation and improve antioxidant potential activities in STZ-induced diabetic mice.

### Pretreatment with rhGLP-1 Recovered the Number and Histological Changes in the Pancreas of STZ-Induced Diabetic Mice

As rhGLP-1 pretreated mice were partially protected from STZ-induced diabetes, islets were counted and histologically assessed in mice from each of the three groups (Figure 4). Islet number was radically decreased in Group II (STZ) *versus* Group I (control) mice and Group III (pre-GLP-1 + STZ) mice (Figure 4(D)).

**Table 1.** MDA, SOD, and GSH-PX levels in plasma of Group I, II, and III mice

| | Control (I) | STZ (II) | Pre-GLP-1 + STZ (III) |
|---|---|---|---|
| MDA (mmol/l) | 2.09 ± 0.50 | 3.70 ± 0.50** | 3.00 ± 0.37* |
| SOD (U/ml) | 178.56 ± 13.44 | 176.29 ± 11.06 | 197.64 ± 21.28* |
| GSH-PX (mg/l) | 304.23 ± 48.89 | 261.38 ± 23.36 | 303.61 ± 22.13* |

Each value represents mean ± SEM ($n = 8$ mice per group).
* $P < 0.05$ when compared to Group II (STZ) mice.
** $P < 0.05$ when compared to Group I (control) mice.

**Table 2.** MDA, SOD, and GSH-PX levels in pancreas of Group I, II, and III mice

| | Control (I) | STZ (II) | Pre-GLP-1 + STZ (III) |
|---|---|---|---|
| MDA (mmol/l) | 3.04 ± 0.47 | 6.07 ± 0.85**** | 3.25 ± 0.82** |
| SOD (U/ml) | 199.43 ± 10.52 | 169.07 ± 27.85*** | 217.98 ± 15.40* |
| GSH-PX (mg/l) | 340.68 ± 32.95 | 283.10 ± 28.57*** | 304.11 ± 48.31*** |

Each value represents mean ± SEM ($n \geq 5$ mice per group).
* $P < 0.05$, ** $P < 0.001$ when compared to Group II (STZ) mice.
*** $P < 0.05$, **** $P < 0.001$ when compared to Group I (control) mice.

Furthermore, the cytoplasm and nuclear in the islets of Group II (STZ) mice appeared lytic and shrunken (Figure 4(B)); these histolopathological findings were consistent with other reports of STZ-induced diabetic mice [15]. The histological changes in islets were significantly less apparent in Group III (pre-GLP-1 + STZ) mice pretreated with rhGLP-1 before STZ (Figure 4(D)). These results demonstrated that rhGLP-1 pretreatment protected against islet damage and may mitigate islet loss in STZ-induced diabetic mice. These findings are consistent with the improved blood glucose levels, glucose tolerance, and diabetic symptoms observed in Group III (pre-GLP-1 + STZ) mice relative to Group II (STZ) mice.

## Discussion

The evidence from our previous study demonstrated that a GLP-1 analog (mGLP-1) protected neuronal SH-SY5Y cells



  

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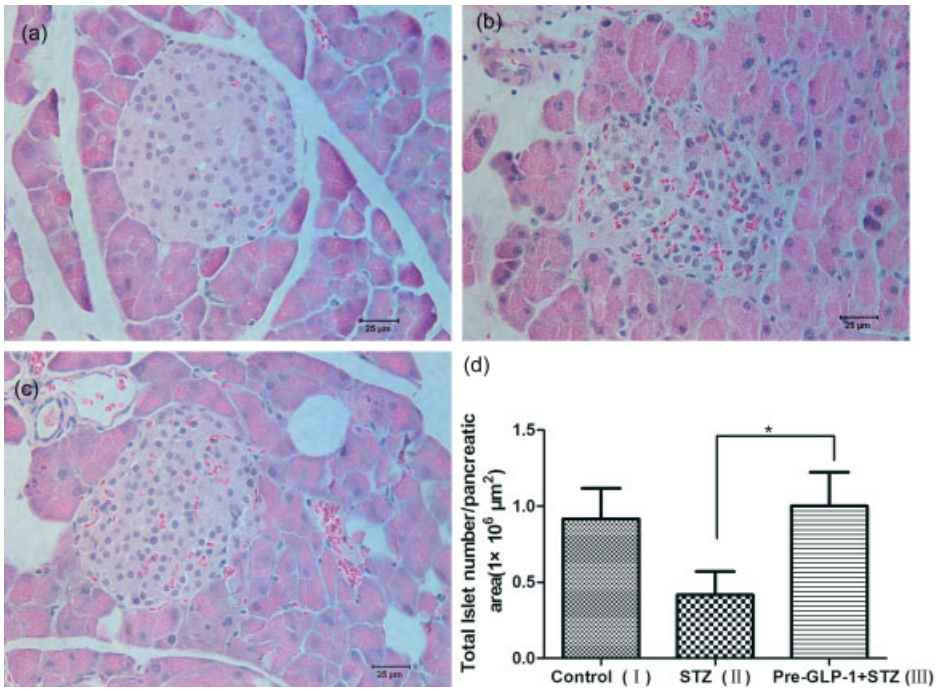


**Figure 4.** Effects of rhGLP-1 treatment on histology and islet number. (a) Representative pancreatic islet of Group I (control) mice. (b) Representative pancreatic islet of Group II (STZ) mice showing depletion of islets and uneven distribution of cell nuclei. (c) Representative pancreatic islet of Group III (pre-GLP-1 + STZ) mice, showing normal islets. (d) Islet numbers in the pancreas of mice in Groups I, II, and III; the number of islets relative to the total pancreatic area examined is shown. $^*P < 0.05$ as compared between Group II and Group III mice.

from apoptosis induced by amyloid-beta peptide [7]. Hui's [8] group showed GLP-1 protected insulinoma MIN6 cells from apoptosis induced by oxidative damage ($H_2O_2$) but was unable to rescue cells from apoptosis once such damage had proceeded. They demonstrated that GLP-1 protected cells from $H_2O_2$ damage through upregulating Bcl-2 family proteins, which was independent of the effects of GLP-1 on glucose control and insulin secretion. In this study, we treated mice with rhGLP-1 before or after STZ injection to investigate the protective effect of rhGLP-1 on STZ-induced diabetes *in vivo*.

STZ is a standard chemical used to induce diabetes mellitus in experimental animals. The cytotoxic action of STZ is associated with the generation of ROS that cause oxidative damage to pancreatic beta cells [16]. In this study, we used two independent experimental approaches: (i) mice pretreated with rhGLP-1 before STZ administration and (ii) mice treated with rhGLP-1 after STZ administration. We found that KM mice treated with STZ 40 mg/kg body weight for 3 days was capable of inducing increase of blood glucose, lipid peroxidation, and pancreas islet cell damage and decrease of antioxidant defense system. In contrast, pretreatment with rhGLP-1 rescued these phenotypes in STZ-induced diabetic mice. However, treatment with rhGLP-1 after STZ administration did not rescue any of the STZ-induced diabetic phenotypes. These results suggest that pretreatment of rhGLP-1 has a protective effect on STZ-induced oxidative stress and cell damage.

It is well known that GLP-1 acts as a potent incretin that can lower postprandial elevated blood glucose and inhibit glucagon secretion [17,18]. However, because of its rapid elimination by DPPIV, unmodified rhGLP-1 is ineffective in the clinical setting [11,19]. After subcutaneous injection of the maximally tolerable GLP-1 dose (1.5 nmol/kg) in patients with type 2 diabetes, there is only a small glucose-lowering effect on plasma glucose and a small short-term positive effect on insulin secretion [11]. Green *et al*. [20] showed that treatment of adult *ob*/*ob* mice with native GLP-1 for 21 days did not lead to a significant effect on plasma glucose, glucose tolerance, islet area, or islet number. These results are also consistent with this study, which showed that Group V (STZ + post-GLP-1) mice injected once-daily with STZ on days 1–3 followed by once-daily 24 nmol/kg rhGLP-1 treatment on days 7–17 did not exhibit lower plasma glucose levels compared with untreated STZ-injected Group IV (STZ) mice (Figure 1(B)). Therefore, these findings suggest that the protective effect of rhGLP-1 (24 nmol/kg) on STZ-induced diabetic mice is independent of the effects of GLP-1 on glucose control and insulin secretion.

In this study, we found that pretreatment with rhGLP-1 before STZ injection but not after had a protective effect against STZ-induced diabetic mice. This finding was demonstrated by reduced blood glucose levels, improved glucose tolerance, and improved islet cell morphology in Group III (pre-GLP-1 + STZ) mice. Our data are corroborated by a study by Hui *et al*. [8], which showed that GLP-1 treatment of cells that were previously exposed to hydrogen peroxide failed to prevent apoptosis, whereas pretreatment with GLP-1 before hydrogen peroxide exposure had a significant protective effect on cell death. They demonstrated that GLP-1 had a direct anti-apoptotic effect on MIN6 cells by increasing expression of anti-apoptotic protein Bcl-2 via a cAMP- and PI3K-dependent signaling pathway [8]. We also measured Bcl-2 mRNA level in pancreas tissues (Supporting Information Figure **S**1). We found Bcl-2 mRNA was significantly decreased in STZ-induced diabetic mice compared with saline-treated control mice, whereas Bcl-2 mRNA in pre-GLP-1 + STZ mice was at the similar level as in the control mice. These results suggest that rhGLP-1 may antagonize apoptosis by enhancing the expression of anti-apoptotic protein Bcl-2. As a result, the number and morphology changes in the pancreas islets in STZ-induced diabetic mice were rescued by rhGLP-1 pretreatment.



  

In addition to the effect of GLP-1 on cell damage, studies of STZ-induced oxidative stress showed that MDA levels of plasma and pancreas were significantly reduced in pre-GLP-1 + STZ group mice relative to untreated STZ-induced diabetic mice. Furthermore, SOD and GSH-PX activities were either enhanced or restored in rhGLP-1-pretreated STZ-induced diabetic mice. These results indicate that rhGLP-1 may decrease oxidative stress levels through increasing antioxidant defense system activities. As rhGLP-1 has a protective effect on pancreas islet cells, the decreased oxidative stress may be partially due to the induction of anti-apoptotic proteins induced by rhGLP-1.

In summary, our results suggest that GLP-1 shows a powerful protective effect on STZ-induced diabetes *in vivo*. Pretreatment of rhGLP-1 inhibited the increase of oxidative stress and pancreas cell damage that are induced by STZ. As diabetes mellitus is a disease characterized by loss of beta cell function and increased oxidative stress, understanding the protective effect of rhGLP-1 in the pancreas islet cells is potentially relevant to the prevention and therapy of diabetes. Our findings of rhGLP-1-mediated protection against STZ-induced diabetes warrant further investigation on optimizing the protection effect of rhGLP-1 on pancreas through pharmacokinetics studies such as does-dependent and time-dependent effect of rhGLP-1 pretreatment.

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### Supporting information

Supporting information may be found in the online version of this article.

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